Attorney Docket No. 108907-09014

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
R. PORTA et Al.	)	Examiner: G. Kishore
Serial No. 09/330,215	)	Art Unit: 1615
Filed: June 11, 1999	)	
For: USE OF COMPLEXES LIPOSOMES AND POLYDEOXYRIBONUCLEOTIDES AS MEDICAMENTS	)	

DECLARATION OF DR. ROBERTO PORTA

PURSUANT TO 37 C.F.R. § 1.132

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, ROBERTO PORTA, do hereby declare that:

2. I am one of the Inventors of the present Application.

3. Under my supervision the following experiments were performed.

Analytical methods

- Molecular weight

Molecular weight was determined using a low angle laser light scattering Chromatix® equipment. Samples were prepared by dissolving the compound to be tested in phosphate-EDTA buffer 0.013 M pH 6.8 at different concentrations in the range from 1

to 5 mg/ml. Reading of the diffused light (P_θ) and of the transmitted light (P_0) of each sample and of the buffer were taken.

The Rayleigh ratio R_θ was then calculated from the equation : $R_\theta = P_\theta/P_0 x K$, wherein K is corrective factor depending on the instrument used.

For each concentration the ratio K_c/R_θ was calculated, wherein $K_c = 2\pi^2/(N\lambda^4) x n^2 x (dn/dc)^2 x (1+\cos^2\theta)$, wherein n is the refractive index, dn/dc is the differential refractive index, θ= solid ring for the determination of diffused light intensity (6-7°). λ = wavelength of the laser beam used ($632.7 x 10^{-7}$ cm), N is the Avogadro number. The calculated ratios K_c/R_θ are plotted against the correspondent sample concentrations. It is obtained a straight line which intercept with ordinate axis is $1/M_w$, i.e. the reciprocal of the molecular weight.

A. Depolymerization of nucleic acids

The procedure described in ex. 4, col. 9 of USP 4,985,552 (enclosed), was followed.

Experiment A.1 (Comparison)

Depolymerization to obtain a polydeoxyribonucleotide having a molecular weight above the present limints (7,000-60,000 Da)

30 g of a commercial DNA sodium salt were dissolved in 500 ml of 3 M acetate buffer. The solution was filtered and heated under stirring to 75°C, to depolymerize the nucleic acid.

After 2 hours depolymerization was stopped by adjusting the pH to the value of 8 with 5 N sodium hydroxide. Then heating to 80°C for 60 minutes was carried out. At the end the solution was filtered. 1 volume of said solution was added of 1.5 volumes

ethanol. It was formed a precipitate which was recovered, washed and dehydrated with ethanol, thereafter dried under vacuum at 60°C. 17 g of polydeoxyribonucleotides were recovered.

Light scattering determination showed that the molecular weight of the depolymerized polydeoxyribonucleotide was of 90,000.

Experiment A.2

Depolymerization to obtain a polydeoxyribonucleotide inside the limits of the invention

The same procedure of exp. A.1 (comp.) was repeated, starting from the same high molecular weight commercial DNA, but stopping depolymerization after 3 hours.

13 g of a depolymerized polydeoxyribonucleotide were obtained.

Light scattering determination showed that the molecular weight of the depolymerized polydeoxyribonucleotide was of 50,000.

B. Complexation with liposomes of the depolymerized polydeoxyribonucleotides obtained in exp. A.1 (comp.) and exp. A.2, respectively

Liposomes were prepared as described in example 1 on page 14 of the spec.

3 aliquots of 50 mg of each of each of the two depolymerized polydeoxyribonucleotide preparations were respectively dissolved in 5 ml of isotonic physiologic solution, as described in ex. 1 of the spec, and thereof liposome complexes prepared with the procedure therein described, obtaining opalescent emulsions.

Therefore for each depolymerized polydeoxyribonucleotide

preparation three samples of the liposome complex in water have been prepared.

The prepared liposome complex samples were immediately transferred in air-tight containers and maintained in the dark at the constant temperature of 25°C.

Stability of the samples was assessed by visual inspection according to the criteria set out in the period bridging pages 3-4 of the Spec : the presence of a precipitate indicated instability.

Visual inspection was carried out on the seventh day after liposome complex preparation, and then at the end of each week counted from said seventh day. Results are reported in the following Table 1.

4. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willfull false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willfull false statements may jeopardize the validity of the application or any patent or registration issuing thereon.

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Date July 9, 2003

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Table 1

Stability test at 25°C in water of the liposome complexes with depolymerized polydeoxyribo nucleotides having M.W. 90,000 (outside the present limits), and with depolymerized polydeoxyribonucleotides having M.W. 50,000 (within the limits), respectively. Criteria for evaluating stability was visual inspection. The presence of a precipitate not redispersible in the aqueous phase indicated that the complex was no more stable. In the Table the expression : "depolymerized polydeoxyribo-nucleotides" has been abbreviated into : "dep. pdn."

Liposome Preparation	Appearance of the liposome complex aqueous phase		
	Immediately after preparation	after two weeks	after three weeks
Liposome complex of dep. pdn. M.W. 90,000	opalescent no precipitate	opalescent no precipitate	opalescent presence of a precipitate
Liposome complex of dep. pdn. M.W. 50,000	opalescent no precipitate	opalescent no precipitate	opalescent no precipitate